

## ABSTRACT

Salinity and drought are the two major environmental constraints that affect plant growth and in turn crop productivity, a problem that has been increased by improper land usage and irrigation practices. Thorough understanding of the salt tolerance adaptations of plants in terms of biochemical and molecular levels can provide tremendous knowledge about their tolerance strategies. Salinity stress in plants induces the formation of reactive oxygen species (ROS) and plants have formulated an array of ROS scavenging system (enzymatic and nonenzymatic antioxidants) to mitigate the cellular components from oxidative burst.

Mangroves are intertidal plants inhabiting the coastal zones of tropical and subtropical regions and possess unique characteristics of tolerating high-salinity of seawater and also tidal currents. Recent progresses in understanding the physiological mechanism of salinity adaptation in mangroves on molecular level indicate that the phenomenon is indeed tightly linked to the regulation of gene expression. Recent studies on salinity tolerance in mangroves like *Avicennia marina*, *Aegiceras corniculatum*, *Bruguiera gymnorhiza*, *Bruguiera cylindrica*, *Ceriops tagal*, *Acanthus* species have identified number of differentially expressed stress regulated genes. *Rhizophora apiculata* Blume. is a highly salt tolerant mangrove species inhabiting the coastal belts of Kerala. The present work proposes to examine the ROS cycle and also gene expression in *Rhizophora apiculata* in response to NaCl stress by suppression subtractive hybridization and gene cloning analysis.

Salt stress was imposed on *R. apiculata* seedlings with five different concentrations of NaCl (0, 100, 200, 300, 400 and 500 mM) and also with time slots such as 3, 6, 12 and 24 h durations. NaCl induced remarkable changes in total protein levels and is visualized with protein banding by SDS-PAGE. NaCl

stress induced the profile of superoxide anion and hydrogen peroxide content. Further, the activities of NADPH oxidase, superoxide dismutase (SOD), catalase (CAT) and peroxidase (GPOX) showed varied levels of expression with concentrations and durations. Different isoenzyme of SOD, CAT and GPOX were detected with the NaCl treatment. Enhancement in antioxidant enzyme activities could be an induced response against cellular damage due to NaCl stress. Significant ascorbate-glutathione cycle (in terms of activities of ascorbate peroxidase (APX), regenerating enzymes and isozymes of APX) supports the efficiency of the mangrove to detoxify the ROS hydrogen peroxide from cells. Ascorbate, reduced glutathione and  $\alpha$ -tocopherol increase the capacity of the species to neutralize the toxic effects of NaCl stress.

The molecular part includes Subtractive hybridization using PCR Select<sup>TM</sup> Subtraction kit (Clontech, CA, USA). Several stress genes were isolated, which were then classified to nine functional categories viz., Metabolism, Protein degradation and folding, Secondary metabolism, Cell rescue and defense, Transport facilitation, Signal transduction, Transcription and translation, Photosynthesis and Unclassified genes of unknown function. The expression patterns of twelve of the genes thus obtained were studied at 6 h, 12 h and 24 h time points of salinity stress using Real time PCR. Most genes were found to be upregulated under salt stress and showed maximum upregulation at the 6 h time point. Two of the genes studied were down regulated after 6 h, implying that the pattern of gene expression varies with time of application of stress.

The most important gene identified by SSH and upregulated in Real time RT PCR is Cytochrome P450 monooxygenase (S)-N-methylcoclaurine 3'-hydroxylase (CYP80B3), which acts in the hydroxylation of (S)-N-Methylcoclaurine to yield (S)-3'-Hydroxy-N-methylcoclaurine. The full length gene (1.5 Kb) was isolated and its expression *in planta* was attempted. The

promoter sequence of the gene was also isolated by genome walking approach. The size of the amplified product was ~0.5Kb. Several domains were identified in the promoter region such as CAAT box, TATA box, Myb Binding Site (MBS) etc.

Another gene that was highly upregulated in the real time PCR analysis was that of Hevamine A precursor. The gene (1.1Kb) was isolated, cloned into a binary vector, pMDC 139 and expressed in onion epidermal cells. Syringe infiltration of the binary vector construct into onion peels showed blue colour due to gus activity.

The present results suggest that the synergistic effect of different ROS-scavenging enzymes and compounds effectively improve the biochemical caliber of plants, thereby reducing the degree of oxidative injuries. Similarly, the genes and the gene elements thus isolated may be used to confer the trait of salt tolerance to non – tolerant genotypes, which can ultimately prove beneficial for crop improvement programmes.

**Key words:** Antioxidant enzymes, Mangrove, NaCl, Oxidative stress, Reactive oxygen species, *Rhizophora apiculata*, Salinity, Stress genes